University of Pittsburgh Institutional Biosafety Committee
Guidance on Biosafety Level Assignment for Adeno-Associated Virus (AAV)

Approved June 13, 2011
Revision approved November 10, 2014
Revision approved December 14, 2015
Revision approved March 14, 2016
Revisions approved April 9, 2018

Background

A summary of the recommended Biosafety Containment Levels (BSL) are found on the chart located on the last page. The AAV guidance was adopted by the IBC on June 13, 2011, and revised as new information has been brought forward.

Adeno-associated virus (AAV) and recombinant adeno-associated virus (rAAV) are commonly used for gene expression with fewer associated biosafety concerns when compared to viral vectors that are persistent and able to integrate into the genome. The following is a brief synopsis of IBC guidance relevant to biosafety with respect to AAV/rAAV vectors.

Historically, the IBC has assigned all work with AAV/rAAV to Biosafety Level 2 or Animal Biosafety Level 2 (BSL/ABSL-2). Although the *NIH Guidelines* does list AAV as a risk group one agent, it is under the CDC/OSHA regulations (Blood borne Pathogen Standard *CFR 29: 1910-1030*) that the committee ruled. The rationale behind the stance of the IBC was that AAV are typically "grown" in HEK 293 cells, and therefore infectious "risk" from the human cell line proponent is considered to exist.

The IBC convened an AAV focus group on May 26, 2011, to discuss criteria for which AAV/rAAV could be safely handled at Biosafety Level 1 or Animal Biosafety Level 1 (BSL/ABSL-1). The guidance was first adopted by the IBC on June 13, 2011, and has been revised to include additional information or knowledge.

NIH opinion

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* identify AAV types 1-4, and rAAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product (for example, an oncogene) or a toxin molecule, and are produced in the absence of a helper virus as risk group 1 (RG1) agents, which are not associated with disease in healthy adult humans (see *Appendix B-1* of *the NIH Guidelines*).

IBC Guidance

The University of Pittsburgh IBC will apply *all* of the following criteria for determining the appropriate biosafety containment and handling of AAV/rAAV:

- Propagation with or without helper virus, including the use of adenovirus
- Presence of transgenes encoding oncogenes or toxins
- Propagation in insect cell lines versus human cell lines
- Verification of the purification techniques and quality control assays used when propagation of virus occurs in human cell lines

IBC Rationale

The OSHA Blood borne Pathogen (BBP) Standard (29 CFR 1910.1030) requires that institutions minimize exposure of research personnel to blood borne pathogens. In accordance with the OSHA standard, pathogenic microorganisms that are present in human blood, human body fluids, human tissues, or other *potentially infectious material, including recombinant or synthetic nucleic acid molecules* are identified in the University of Pittsburgh Blood borne Pathogens Exposure Control Plan to fall under the BBP Standard.

Adeno-Associated vectors that are grown in insect cell lines with no helper virus may be used under BSL-1/ABSL-1 conditions without additional purification, but many AAV vectors are grown in human cells or cell lines.

The IBC requires the purification to remove any potential human pathogens in the final vector. The purification steps need to be verified to confirm that the procedures were effective and that the resultant vector is reliable. Quality Control analysis by SDS page or silver staining is an acceptable method used to verify the purity of the viral preparation.

This is important for safety issues, but it is also recognized to be an important factor in experimental reproducibility and reliability for grant applications.

BSL/ABSL-1: Specific requirements for Use of AAV/rAAV

The IBC will consider designating adeno-associated viruses or recombinant adeno-associated viruses for use at BSL/ABSL-1 if the following *three* criteria are met:

1. Transgene does not express an oncogenic protein or toxin (see: ***NIH Guidelines* reference** Section III-B-1)
2. AAV/rAAV is generated *without* using adenovirus *or* any other helper virus of human origin (this includes helper plasmids per the NIH Office of Biotechnology Activities)
3. AAV/rAAV is propagated in insect cell lines

Determination of the biosafety level for AAV/rAAV meeting criteria *1* and *2* above *and* yet are propagated in human cell lines will be made by the IBC when all of the specific requirements have been addressed (see below "*Exceptions to the requirement for BSL/ABSL-2*").

BSL/ABSL-2: Specific requirements for Use of AAV/rAAV

Adeno-associated viruses or recombinant adeno-associated viruses ***must*** be used under BSL/ABSL-2 containment if:

1. Transgenes express an oncogenic protein or toxin
2. Helper virus of human origin is used to generate AAV/rAAV
3. AAV/rAAV is propagated in human cell lines ***without*** further purification before use

*Exceptions to the requirement for BSL/ABSL-2

AAV/rAAV are typically propagated in HEK 293 cells, a commercially available human cell line. Under the Code of Federal Regulations (*29 CFR 1910-1030*) otherwise known as the Blood Borne Pathogen Standard, all human-derived materials are to be handled under BSL-2 (Universal Precautions) conditions, per CDC and OSHA regulations.

The IBC will consider reducing the biosafety level to BSL/ABSL-1 when the following criteria

are met and documented in the IBC protocol application. These additional requirements are in addition to the oncogene/toxin expression and helper virus criteria detailed above:

A) AAV/rAAV generated in non-human cells, or AAV/rAAV generated in human cells by a helper virus-free plasmid transfection method with subsequent purification and appropriate quality control

The investigator must provide details of the techniques used for purification and quality control on the IBC application for the following three criteria:

1) Purification

For example: cesium chloride or iodixanol gradient, and/or column chromatography

Purification is valuable for authentication of biological agents with qualities or qualifications that could impact research data and reproducibility.

2) Quality control

For example: SDS-PAGE gel electrophoresis, Western blot and/or silver stain to assure appropriate purification.

The figure below (*J. Gene Med 2004; 6: S223-S228*) demonstrates the use of purification and quality criteria described above for generation of two FPLC-purified AAV vectors. In both examples (A and B) the first column represents pre-purification viral preparation, the second and third columns represent purification fractions collected after column separation.

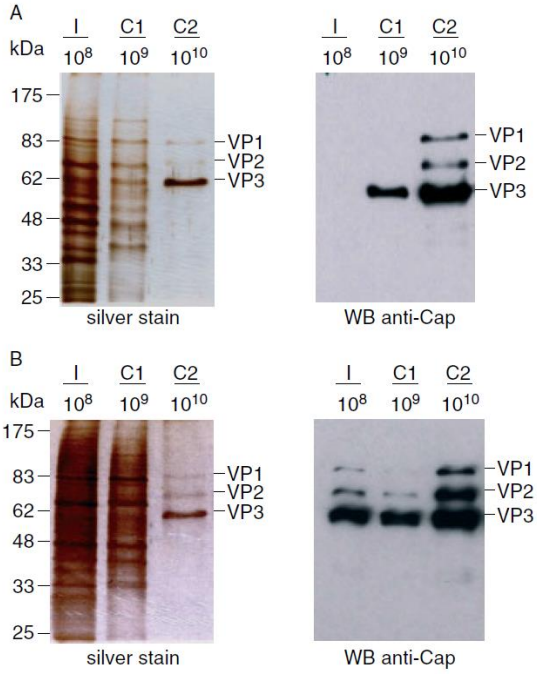

Figure 3. Silver staining and Western blot analysis of FPLC-purified rAAV-2 (A) and -5 (B) particles. Samples containing known amounts of rAAV particles (measured by dot blot) and indicated on the top of each lane were resolved by SDS-PAGE and either silver-stained or transferred to a membrane and analysed by Western blot to detect AAV Cap proteins. I: input cell lysate; C1: pool of the rAAV-containing fractions collected after the first chromatography step; C2: pool of the rAAV-containing fractions collected after the second chromatography step [37]

Gradient columns are often reused, therefore quality control analysis plays an

important role in assessing safety as well as demonstrating the purity of the experimental vector.

The IBC threshold for AAV vector purity is 80% and above. Less than 80% purity may indicate other potentially infectious material (OPIM) in the vector, and will require additional safety precautions.

3) Record maintenance
Investigators requesting downgrade for specified AAV vectors at biosafety containment level (BSL/ABSL-1) are required to keep the quality control data (criteria b) in the laboratory records, in accordance with University policy.

B) AAV vectors obtained from core facilities that perform both purification *and* quality assurance testing of the batch lot *prior* to distribution are recommended by the IBC, because such AAV vectors contribute to experimental reproducibility and reliability, and ***may be eligible*** for work under BSL/ABSL-1 containment.

The table below provides information on recognized core facilities production practices:

Table 1 Recognized Core Facility production summary

Recognized AAV production Core Facilities		
Core	**Purification Procedure**	**BSL**
UNC	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE/Silver Stain per vector per lot Will provide purity and titer per lot	Automatic BSL-1
MWRI	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE/Silver Stain per vector per lot Will provide purity and titer per lot	Automatic BSL-1
Addgene	Iodoxinal Gradient followed by concentration QC analysis by qPCR titer, SDS-PAGE/Silver Stain Will provide results of QC upon request	Automatic BSL-1
Salk Institute (CA)	Purification on a discontinous OptiprepTM gradient; price per prep. Custom rAAV preps are titrated using qPCR to give titer in genome copies (GC) per ml	BSL-2 unless purification and QC data provided
Stanford	Provides unpurified AAV unless otherwise requested Core facility recommends use under BSL-2	BSL-2 unless purification and QC data provided
U Penn	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE is available upon request Will provide purity and titer per lot at cost	BSL-2; downgrade possible with QC data provided

Note that there are Core facilities that perform purification and quality control analysis on *all* batch lots of viruses or vectors. The quality control data is available at no additional charge, but the data must be requested for a specific lot of vector. Usually this is provided as a "COA" or Certificate of Analysis.

Other Core facilities may perform purification, but do not specifically provide quality control analysis on all batch lots unless this verification step is requested for a fee.

C) Investigators who are not generating their own viruses but are acquiring viruses from another University of Pittsburgh laboratory to which the IBC has previously granted approval to use AAV/rAAV at BSL/ABSL1, should provide the IBC registration number(s) and the name of the University of Pittsburgh investigator(s) providing AAV/rAAV. In this case, detailed description of the method used for generating, purification, and quality control methodology may be omitted from the application.

Table 2 summarizes the Institutional Biosafety Committee's position on Biosafety Levels (BSL) for work with AAV:

Table 2 Summary of Biosafety Containment Levels

Summary of biosafety level requirements for AAV/rAAV use				
Oncogene or Toxin	Human origin Helper Virus is used (e.g. human adenoviruses and herpesviruses)	Propagated in Human Cell Lines (e.g. HEK 293 cells)	**Purification and Quality Control	Recommended BSL/ABSL
Yes	Yes	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
	No	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
No	Yes	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
	No	Yes	Yes	1
			No	2
		No	Yes	1
			No	1

****NOTE on Purification and Quality Control**: The purification assurance (a.k.a. Quality Control or Quality Assurance) step may not be offered as a standard ordering item, and may have to be specifically requested by the researcher and therefore may incur additional product fees or pricing when purchased or obtained from a commercial vendor or another University's Vector Core. See Table 1 for source facility information.